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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 15 March 1999 with an application for Letters Patent number 334664 made by UNIVERSITY OF OTAGO;THE MALAGHAN INSTITUTE OF MEDICAL RESEARCH.

Dated 21 March 2000.

Neville Harris
Commissioner of Patents

334664

Patents Form No 4

Patents Act 1953

Provisional Specification

A VACCINE

We, **The Malaghan Institute of Medical Research,** a non-profit organisation, of Mein Street, Newtown, Wellington, New Zealand do hereby declare this invention to be described in the following statement:

This invention relates to an improved method for reducing the severity of asthma and/or the risk of developing asthma.

BACKGROUND ART

Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, including mast cells and eosinophils. In susceptible individuals this inflammation causes symptoms which are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment, and causes an associated increase in airway responsiveness to a variety of stimuli.

Asthma can be inherited, is not contagious and may be chronic and persistent or occurring in the form of attacks which are periodic and usually at least partly reversible. Attacks vary in severity and frequency from person to person. Many factors may contribute to the development of asthma including exposure to inhaled allergens such as pollens, mold spores, house dust mites and animal dander. In an individual who has developed asthma, many stimuli can trigger asthma attacks including allergens, viral respiratory infections (colds or the flu), irritants in the air (smoke, air pollution, perfume), damp, cold weather, and strenuous exercise. In addition, anxiety and emotional stress can bring on and worsen the symptoms of asthma.

During an asthma attack the muscles around the bronchial tubes tighten and the linings of the bronchial tubes swell (become inflamed) and produce thick mucus decreasing the internal diameter of the tubes. These changes block the flow of air making it hard to breathe. When asthma is properly controlled the bronchial tubes are of normal size.

Asthma is a common disease among both children and adults. An estimated 7% of people in the United States have been diagnosed as asthmatic. The corresponding figure for New Zealand is about 10% (Burney, P. et al. 1996 Variations in the Prevalence of Respiratory Symptoms, Self-Reported Asthma Attacks, and Use of Asthma Medication in the European Community Respiratory Health Survey. *Eur. Respir. J.* 9:687-695). The occurrence of asthma in both Western and developing countries has increased markedly over the last 30 years. This relatively short time frame suggests that environmental rather than genetic factors are at work.

In most cases asthma is an atopic disorder in which the underlying process is due to an allergic response to common environmental allergens. This allergic response is a function of the immune system characterised by activation and recruitment of eosinophils to the lung causing the characteristic chronic swelling and inflammation of the airways that affects the breathing of sufferers.

The pharmaceutical treatment of asthma includes several different classes of drugs, including beta agonists, topical or oral steroids and theophyllines. If used appropriately such treatments may keep asthma symptoms from developing or relieve them when they are present. Beta agonists and theophyllines primarily act by relaxing the muscles surrounding the airways while steroids act to reduce (and even prevent) inflammation and mucus production. Other medications exist and more are being developed due to the growing interest in and concern over the prevalence, morbidity and mortality of asthma worldwide.

Asthma is known to be a disease of the immune system. It is known that the process leading to inflammation of the airways is mediated by the Th2 lymphocytes (Th2s) whose usual function is to act against parasites. These cells secrete the cytokines interleukin-4 (IL-4) and IL-5 leading to enhanced production of immunoglobulin E (IgE) by B cells and the generation and recruitment of eosinophils respectively. Activation of mast cells by allergens releases histamine and other mediating chemicals that trigger an acute inflammatory response, including mucus production intended to flush dying parasites off tissue walls. In asthma the enhanced mucus production merely hinders breathing further. Eosinophils release cytotoxins meant to kill invading parasites but in asthma merely result in inflammation and necrosis of the bronchial epithelium. The increased eosinophil population, localised recruitment and the resultant tissue damage is termed "eosinophilia".

The global increase in atopic disorders such as asthma, hay fever and eczema is inversely correlated with the decline in the extent of exposure to human diseases such as tuberculosis, measles and whooping cough. These diseases all illicit a characteristic Th1 type immune response, mediated by the Th1 lymphocytes (Th1s), which leads to heightened expression of the cytokine interferon-γ (IFN-γ), a powerful suppressive mediator of Th2 activity. It has been suggested that the lack of exposure to such Th1 inducing infections increases the risk of developing atopy and that by inducing a Th1 type immune response it is

possible to down regulate the Th2 response thus reducing the likelihood of developing atopic disorders such as asthma.

The need exists for an asthma treatment that modulates the immune system to reduce the risk of developing atopy, rather than the traditional treatment of responding with drugs to suppress or alter the symptoms once the disorder has developed or the sufferer is in the midst of an attack. An added benefit would be if such a treatment also has a similar inhibitory effect in a current sufferer of an atopic disorder to reduce the severity of their disease.

PRIOR ART

An article entitled "Relationships Between the Structure and the Roles of Lipoarabinomannans and Related Glycoconjugates in Tuberculosis Pathogenesis" (Vercellone et al 1988 *Froniters in Bioscience* 3, e149-163) discusses the involvement of lipoglycans including lipoarabinomannan (LAM) in mycobacterium pathogenesis. It discloses that lipoarabinomannan (LAM) will modulate the secretion of Tumor Necrosis Factor-alpha (TNF-α). It was propounded that there is a relation between virulence and the capacity of LAM to induce TNF-α production.

US Patent specification 5853737 (Modlin) discusses various methods of inducing a CD1 restricted immune response and teaches of a vaccine containing CD1-presented non-polypeptide hydrophobic antigens and in particular a lipoarabinomannan (LAM) antigen.

Both US Patent specifications 4329452 and 4394502 (Maruyama) teach of the use of lipopolysaccharide as an active component in an immunotherapeutic agent for tumors. The lipopolysaccharide can be derived from human tubercle bacillus.

US Patent specification 5679347 (Porcelli) also teaches of the preparation and use of a vaccine which contains CD1-presented antigens and in particular where the CD1-presented antigen is a mycolic acid.

OBJECT OF THE INVENTION

Therefore it is an object of this invention to provide a method for reducing the risk of the development of asthma in non-sufferers, and for reducing the severity of asthma in sufferers.

SUMMARY OF THE INVENTION

Accordingly one form of the invention may be said to comprise a vaccine for reducing the severity of asthma comprising of an immunologically effective dose of one or more Th1 type immune response inducing substances.

Accordingly a further of the invention may be said to comprise a vaccine for reducing the risk of developing asthma comprising of an immunologically effective dose of one or more Th1 type immune response inducing substances.

Accordingly a further form of the invention may be said to be a method for reducing the severity of asthma comprising

administering a vaccine

consisting of an immunologically effective dose of one or more Th1 type immune response inducing substances

to the airways of individuals.

Accordingly a further form of the invention may be said to be a method for reducing the risk of developing asthma comprising

administering a vaccine

consisting of an immunologically effective dose of one or more Th1 type immune response inducing substances

to the airways of individuals.

Preferably the vaccine is administered to the respiratory tract.

Preferably the vaccine consists of *Mycobacterium bovis* (Bacillus Calmette-Guérin [BCG]).

Preferably the vaccine consists of lipoarabinomannan (LAM).

Preferably the vaccine consists of a purified protein derivative (PPD) of BCG.

Preferably the vaccine consists of alkyl hydro peroxide reductase (AhpC).

Preferably the vaccine consists of the influenza virus.

Preferably the vaccine consists of the *Brucella abortus* antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing number of cells recovered per ml of bronchoalveolar lavage (BAL) exudate.

Figure 2 is a graph showing total number of cells recovered by BAL.

Figure 3 is a graph showing the percentage of eosinophils recovered by BAL.

Figure 4 is a graph showing the percentage of macrophages recovered by BAL.

Figure 5 is a graph showing number of eosinophils recovered per ml of BAL exudate.

Figure 6 is a graph showing total number of eosinophils recovered by BAL.

BEST MODE OF PERFORMING THE INVENTION

The present invention offers a method of reducing the severity of airway eosinophilia and thus asthma and/or for reducing the risk of developing airway eosinophilia and thus asthma by introducing to the airways biologically active amounts of one or more of the following:

(a) dead Th1 immune response inducing *Mycobacterium bovis* (Bacillus Calmette-Guérin [BCG];

(b) lipoarabinomannan (LAM), a major lipoglycan of the mycobacterial cell wall;

(c) a purified protein derivative (PPD) of BCG;

(d) alkyl hydro peroxide reductase (AhpC), an enzyme believed to be a virulence factor important for the survival of mycobacteria within macrophages;

(e) influenza virus;

(f) *Brucella abortus* antigen.

An ovalbumin (OVA) induced airway eosinophilia mouse model of atopic airway inflammation was used to determine the effectiveness of (a) to (f) in suppressing the development of airway eosinophilia. Antigen specific Th2 cells were primed to OVA by two successive intraperitoneal immunisations 14 days apart with OVA and by administration of an intranasal challenge of OVA 7 days after the second intraperitoneal immunisation. Biologically active amounts of (a) to (f) were given intranasally to the primed mice with the first and second OVA immunisations. 3 to 5 days post intranasal challenge bronchoalveolar lavage (BAL) was used to determine the degree of eosinophil inflammatory response. BAL exudates were examined for the presence of eosinophils and for any effect on the development of IL-4 and IL-5 producing Th2 lymphocytes in the draining mediastinal lymph node.

EXAMPLE 1

BCG killed by heating at 56°C for 30 minutes were given to mice at doses of 5×10^5, 5×10^7 and 5×10^8 colony forming unit (CFU) equivalents. The doses were administered either intranasally or subcutaneously and their effect determined by BAL as described above.

EXAMPLE 2

Lipoarabinomannan (LAM) is a major lipoglycan of the mycobacterial cell wall. A potentially important feature of LAM is whether it is capped by arabinose or mannose sugars, with several correlation's being made between the virulence of the mycobacterial species and the degree of LAMs capped by mannose. LAM modulates the secretion of TNF-α and is able to induce the secretion of other cytokines. Doses of 0.1 to 100 μg/ml of LAM were administered to the airways of mice 21 and 7 days before intranasal OVA challenge. Experiments with subcutaneous injection of LAM were also performed. The effect of LAM on airway eosinophilia was determined by BAL.

EXAMPLE 3

Doses of 1, 10 and 100 μg per mouse of PPD were given at 21 and 7 days before OVA challenge. Experiments with subcutaneous injection of PPD were also performed. The effect of PPD on airway eosinophilia was determined by BAL.

EXAMPLE 4

Doses of 0.5, 5 and 50 μg per mouse of AhpC were given at 21 and 7 days before OVA challenge. Experiments with subcutaneous injection of AhpC were also performed. The effect of AhpC on airway eosinophilia was determined by BAL.

The above examples are not intended to be any way limiting and are intended to be illustrative only of certain aspects of the invention.

Figures 1 to 6 show the results of several of these experiments. Figures 1 and 2 show the number of cells recovered for each experiment per ml and in total. Figures 3, 5 and 6 show that immunisation with high dose LAM gives rise to a reduction in eosinophil numbers equal to or greater than the reduction seen with whole live or dead BCG. This implies that LAM may be the active component in BCG that suppresses airway eosinophilia. Figure 4 shows that the number of macrophages in mice immunised with whole BCG (live or dead) is equivalent to the number found in mice immunised with high doses of LAM. This supports the observation in the prior art that TNF-α (which activates macrophages) is stimulated by LAM.

Having described preferred methods of putting the invention into effect, it will be apparent to those skilled in the art to which this invention relates, that modifications and amendments to various features and items can be effected and yet still come within the general concept of the invention. It is to be understood that all such modifications and amendments are intended to be included within the scope of the present invention.

McCABE AND COMPANY
By
ATTORNEYS FOR THE APPLICANT

Figure 1

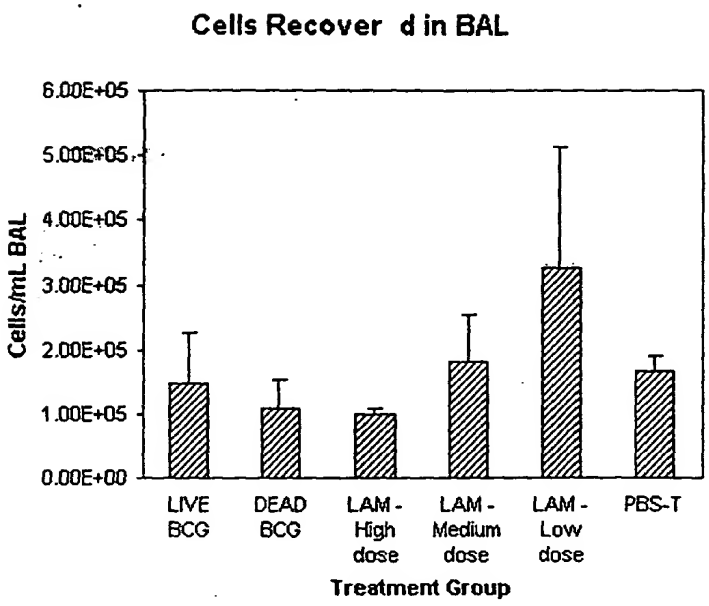

Figure 2

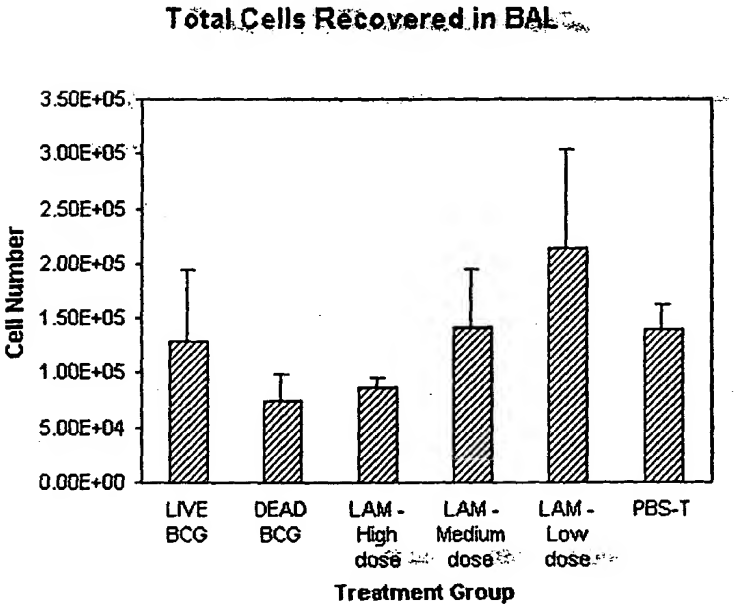

Figure 3

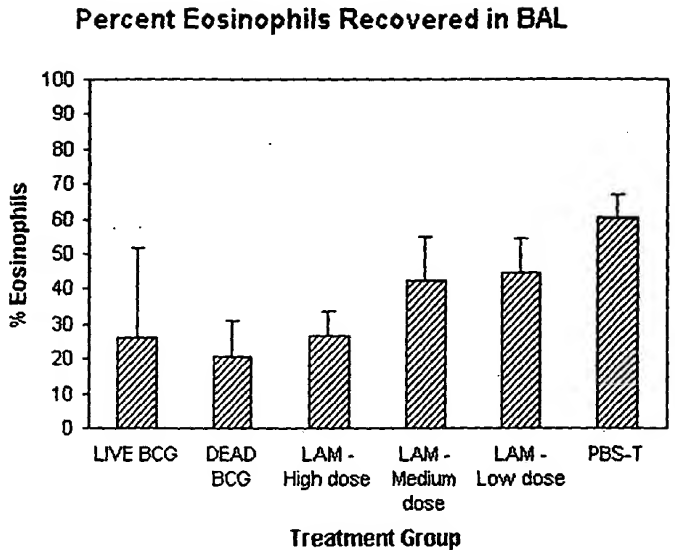

Figure 4

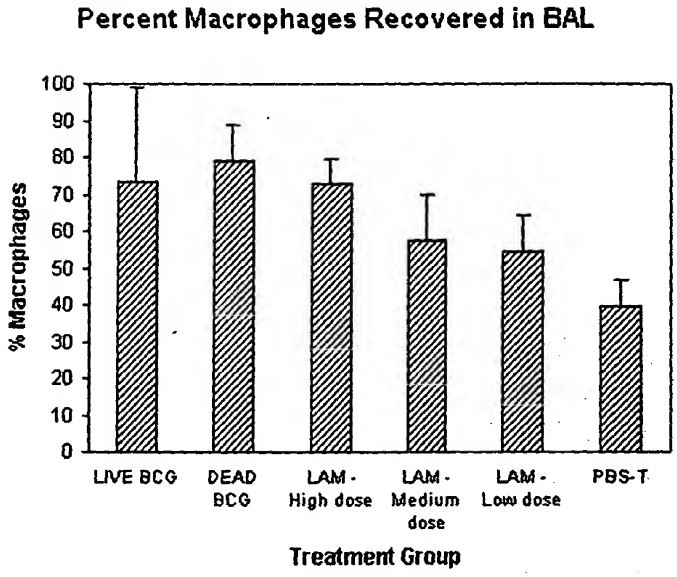

Figure 5

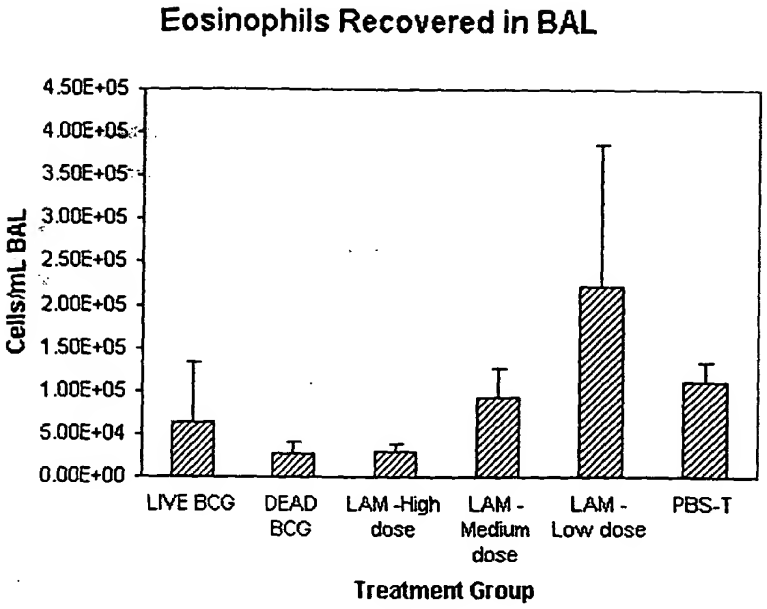

Figure 6